TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2017) 41: 113-120

doi:10.3906/tar-1612-56

Contents of phenolics and carotenoids in tomato grown under polytunnels with different UV-transmission rates

Bojana ŽIVANOVIĆ[1], Marija VIDOVIĆ[1,*], Sonja MILIĆ KOMIĆ[1], Ljubinko JOVANOVIĆ[2], Predrag KOLARŽ[3], Filis MORINA[1], Sonja VELJOVIĆ JOVANOVIĆ[1]

[1]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia
[2]Faculty of Ecological Agriculture, Educons University, Sremska Kamenica, Serbia
[3]Institute of Physics, University of Belgrade, Belgrade, Serbia

Received: 16.12.2016 • **Accepted/Published Online:** 15.03.2017 • **Final Version:** 03.05.2017

Abstract: Tomato (*Solanum lycopersicum* L.) is among the economically most important vegetables in Europe, valued for its bioactive properties due to significant contents of vitamins, carotenoids, and phenolic compounds. In this study, the tomato cultivar Big Beef F1 was grown in the open field (OF) and under polytunnels in central Serbia during 3 years. Polytunnels were covered with two foils (both with 57% reduced photosynthetic active radiation, PAR) differing in UV-A and UV-B transmittance. The aim of our work was to determine the influence of light conditions on accumulation of phytonutrients (carotenoids and phenolics) in the peel and flesh of ripe tomato fruits. The amount of effective antioxidants, caffeic acid, and quercetin (phenolics with *ortho*-dihydroxy substitution) in the peel was the highest in tomato fruits grown in the OF (maximal PAR and UV-A and UV-B radiation). Moreover, the content of leaf epidermal flavonoids was the highest in the OF. The content of lycopene and β-carotene in the flesh of tomato fruit was higher under the polytunnel with higher UV-transmittance. Our results showed that selection of the right light conditions (quality and intensity) for tomato production has a significant effect on the accumulation of beneficial phenolics and carotenoids.

Key words: Phytonutrients, flavonoids, solar radiation, protected cultivation, *Solanum lycopersicum* L., UV radiation

1. Introduction

Numerous reports on the beneficial effects of natural antioxidants for human health have drawn attention to food sources (fruits and vegetables) and means of improving their nutritional value. Tomato (*Solanum lycopersicum* L.) is a high-value crop and one of the most widely grown vegetables (Sabir and Singh, 2013). According to the Food and Agriculture Organization (http://faostat.fao.org), in the period from 2013 to 2014 tomato production in four Mediterranean countries (Turkey, Egypt, Italy, and Spain) ranked second in the world, after China. Moreover, tomato was among the top three vegetables in EU in terms of the level of production (17.6×10^6 t in 2015; Eurostat, 2016). The geographical location of Serbia, with a predominantly moderate continental (north) and Mediterranean (south, southeast) climate, has proved to be suitable for tomato production; in 2012 and 2013 Serbia was in the fourth place in tomato production (135,000–155,000 t/year) in Southeast Europe, after Greece, Romania, and Albania (http://faostat.fao.org).

During the last decade, protected cultivation under polytunnels increased and became a major production system for supplying tomato all year round throughout the world (Peet and Welles, 2005; Boulard et al., 2011; Sabir and Singh, 2013). However, under glasshouses and polytunnels photosynthetically active radiation (PAR, 400–700 nm) intensity is attenuated and most of the UV radiation is excluded (Jansen et al., 2008; Lamnatou and Chemisana, 2013).

Red tomatoes are a rich source of bioactive compounds, such as carotenoids and phenolics. The beneficial effects of carotenoids (lycopene and β-carotene) have been reported with respect to a wide range of diseases and health conditions and have been attributed to their antioxidative and provitamin A activities (Rao and Rao, 2007; Kotíková et al., 2011). In addition, polyphenolics, a large group of secondary metabolites in plants, are the subject of increasing scientific interest due to their importance for human health (Del Rio et al., 2013; Zhang and Tsao, 2016), mostly based on their antioxidative functions (Rice-Evans et al., 1997). In plants, phenolic compounds are involved

* Correspondence: marija@imsi.rs

in many processes, from growth and development to flowering, reproduction, and seed dispersion, and in protection against abiotic stress and pests (Gould and Lister, 2005; Lattanzio et al., 2006). For example, depending on their chemical structure and localization at tissue level (dermal tissues, mesophyll, etc.), phenylpropanoids and flavonoids can act either as ultraviolet (UV) and/or visible light attenuators (screeners), or as efficient antioxidants (Agati et al., 2013).

Although accumulation of secondary metabolites and especially flavonoids and terpenoids in fruits may be determined by internal factors (e.g., genetic variation), it can be triggered by ecologically relevant doses of UV-A (320–400 nm) and UV-B (280–320 nm) radiation (Jansen et al., 2008; Becatti et al., 2009; Schreiner et al., 2014). The final effect of UV radiation on accumulation of phytonutrients depends on the biologically effective dose applied and/or the spectral quality (Giuntini et al., 2005; Avena-Bustillos et al., 2012), as well as on interactions with other environmental factors, such as background PAR intensity (Neugart et al., 2012). Moreover, the synergistic effect of UV-A, UV-B, red, and blue light on the accumulation of phenolics and carotenoids in leaves and fruits has been observed (Ilić et al., 2015; Vidović et al., 2015). Therefore, manipulation of light quality and quantity has opened new possibilities in crop production for increasing the yield, antioxidative, nutritional, organoleptic, and pharmacological value of vegetables, e.g., by increasing the content of carotenoids and phenolics (Luthria et al., 2006; Olle and Viršilé, 2013; Bian et al., 2015).

The aim of our study was to determine how tomato production in the open field and under polytunnels differing in PAR, UV-A, and UV-B transmittance influences the accumulation of lycopene (Ly), β-carotene (β-Car), hydroxybenzoic and hydroxycinnamic acids (HBAs and HCAs), and flavonoids in the flesh and peel of tomato fruits. We hypothesized that different light conditions would alter the amounts and distribution of specific phenolics and carotenoids in red tomato fruits of the cultivar Big Beef F1.

2. Materials and methods

2.1. Experimental site and design

The field experiments were conducted in Svilajnac (44°13′28″N, 21°11′30″E), in central Serbia, on an organic vegetable farm during 2013, 2014, and 2015. Tomato was grown in three different adjacent cropping systems: an open field (OF), and two typical 2-year-old polytunnels. The material, orientation, shape, and dimensions of the polytunnels were the same: 20 m length, 4.5 m width, and 2.5 m maximal height, but covered with two different commonly used polyethylene foils in Serbia: F1 (Tim d.o.o., Banatski Karlovac, Serbia) and F2 (C605, Suncover white, Ginegar Ltd., Kibbutz Ginegar, Israel). Both foils transmitted about 43% of PAR, while UV-A (320–400 nm) and UV-B (280–320 nm) radiation penetration levels were different; UV-B was almost completely excluded by F2 (Table 1). No supplementary lighting or heating was provided under the polytunnels. UV transparency of the covering materials was measured using a PMA 2100 radiometer (Solar Light Company Inc., Glenside, PA, USA) equipped with a UV-A detector (PMA 2110) and UV-B biologically effective radiation detector (PMA 2101). Intensity of PAR was measured using a PAR Quantum Sensor CE (SKP 215 42474; Skye Instruments, Llandrindod Wells, UK). During the 3 years of the experiment we monitored PAR, UV-A, and UV-B transmission properties under the F1 and F2 polytunnels, and no significant changes were observed for F1 or F2.

Each cropping system had two plots with the same size (10 × 4.5 m). The experiment was conducted in a randomized block design with two replicates. All three cropping systems had the same cultivation history and soil properties and were placed on a field that had been under cultivation for at least 50 years before being converted to organic production of vegetables in 2010. The growing substrate in all plots was composed of 70% soil and 30% compost manure, composed of sheep (60%, w/w), pig (30%, w/w), and chicken (10%, w/w) manure. The soil composition (upper layer: 0–30 cm) was $CaCO_3$ (2%–4%), organic matter content (2.84%), and total N content in organic matter (0.14%), while both total P and K contents

Table 1. PAR, UV-A, and UV-B irradiance in three cropping systems (F1, F2, and OF) and transmittance rates (%) of two plastic cover materials (F1 and F2) used in the experiments.

	PAR, UV-A, and UV-B irradiance			Transmittance rate, %	
	OF	F1	F2	F1	F2
PAR (μmol m^{-2} s^{-1})	1816.0 ± 12.9	782.5 ± 19.3	771.5 ± 65.7	43.1 ± 1.1	42.6 ± 3.6
UV-A irradiance, (W m^{-2})	45.2 ± 1.8	14.5 ± 0.1	3.7 ± 0.3	32.1 ± 0.3	8.1 ± 0.7
BE UV-B irradiance, (mW m^{-2})$_{BE}$	163.9 ± 7.8	38.2 ± 2.2	0.3 ± 0.0	23.3 ± 1.4	0.2 ± 0.0

BE, biologically effective.

were higher than 40 mg/100 g. The soil pH value in H_2O was 7.95 and in 1 M KCl 6.84.

Irrigation was done daily, by the drip system, during 1 h in the afternoon, equally for all plots. According to organic cropping management, plants in all plots received only copper sulfate as pesticide, once prior to flowering and the second time prior to fruit formation. Weeds were removed manually when required. The average monthly weather conditions (precipitation; insolation; minimal, maximal, and mean temperature; cloudiness; and relative humidity) from February to July 2013–2015 are presented in Table A1 in Appendix A.

2.2. Plant material and growth conditions

In this study we used the indeterminate Big Beef F1 tomato cultivar, popular among Serbian farmers. Tomato seeds (Seminis, Monsanto Holland BV, Enkhuizen, the Netherlands) were sown in plug trays with a peat/perlite (3:1, v/v) medium in February. Twenty days later, the uniform seedlings were separated and planted in plastic pots for another 30 days. In the first half of April, plants (about 20 cm high) were replanted with uniform spacing (100 cm within the row and 50 cm between rows) to all plots in all three cropping systems within the same day. Six uniform plants per plot, all equally exposed to light, were randomly chosen for the experiments. At the end of June, four to seven healthy, fully light-exposed, red-ripe fruits (RR stage according to Grierson and Kader, 1986) of uniform size per plant were carefully collected by hand at around 1400–1500 hours. Shaded leaves and fruits were carefully excluded from the analyses. Although the average fruit weight varied among the experimental years, fruits from the OF were always about 30% lighter than F1 and F2, while no significant changes were observed between them (data not shown). Temperature at the fruit and leaf surface during sampling was similar in all cropping systems (average: 31.6 ± 1.0 °C). Fruits were washed and wiped, and the flesh and peel (exocarp, approximately 2 mm thick) were excised by razor and separated and samples that originated from the same plant were pooled together. The samples of peel and previously homogenized flesh were freeze-dried and stored at –80 °C for carotenoid and phenolics analysis.

2.3. Epidermal flavonoids and total chlorophyll measurements

Immediately prior to harvest, total chlorophyll content (Chl), content of leaf epidermal flavonoids (EpFlav), and their ratio, the nitrogen balance index (NBI), of the same plants used for fruit collection were measured in vivo with the Dualex FLAV (FORCE-A, Orsay, France; see Cerović et al., 2012 for more details). About ten uniform, fully developed, and fully daily sun-exposed leaves per plant in each plot were analyzed.

2.4. Carotenoid determination

Following homogenization in liquid nitrogen, carotenoids from approximately 0.2 g of dry weight (DW) were extracted according to a modified method described by Davuluri et al. (2005). All samples were extracted in duplicates. The main carotenoids, Ly and β-Car, in pooled extracts (three reextractions) were separated and quantified by HPLC-PDA (LC-20AB Prominence liquid chromatograph, Shimadzu, Kyoto, Japan) using a reversed-phase C18 column (5.0 µm, 250 × 4.6 mm Luna C18 (2); Phenomenex Ltd., Torrance, CA, USA) and isocratic elution gradient composed of 90% methanol and 10% acetonitrile at 25 °C, according to Olives Barba et al. (2006). Ly and β-Car were identified using standards (Sigma Chemical Co., St Louis, MO, USA) and quantified by peak area using Shimadzu LC Solution software (Shimadzu, Kyoto, Japan).

2.5. Phenolics determination

Phenolic compounds were extracted in methanol containing 0.1% HCl and hydrolyzed in 2 M HCl for aglycone determination according to Vidović et al. (2015). All samples were extracted in duplicates. Phenolic compounds were identified and quantified from pooled extracts (three reextractions) using the same HPLC apparatus as for determination of carotenoids. For quantification of flavonoids (chalconaringenin and kaempferol), quercetin was used as standard.

2.6. Statistical analysis

Two-way ANOVA was used to reveal the effects of light conditions (cropping system, CS) and year (Y) and their interactions on the carotenoid and phenolics contents in the peel and flesh of tomato fruits and on the EpFlav, Chl, and NBI. Tukey's post hoc test was used to test for significant differences in the outlined parameters among cropping systems for both tissue types. Both tests were conducted with IBM SPSS statistics software (Version 20.0, IBM Corp., Armonk, NY, USA). The significance threshold value was set at 0.05.

3. Results and discussion

3.1. Chlorophyll and epidermal flavonoid content in the leaves

In order to monitor the fitness of tomato plants, we measured total Chl and EpFlav contents and their ratio, NBI, which is an indicator of C/N allocation changes due to stimulation of flavonoid metabolism under different ambient light conditions (Tremblay et al., 2012). Leaf Chl content was higher in the plants grown in the OF compared with F1 and F2 in 2013 and 2015, while the opposite was observed in 2014 (Table 2; significant effects of CS and Y and their interactions are given in Table B1 in Appendix B).

Table 2. Total chlorophyll (Chl) content, epidermal flavonoid (EpFlav) content, and NBI index in the leaves of tomato grown in OF and under two polytunnels (F1 and F2) during 2013, 2014, and 2015.

Year and cropping system	Chl, mg cm^{-2}	EpFlav, g cm^{-2}	NBI
2013			
OF	36.3 ± 0.4 [b]	0.93 ± 0.02 [c]	39.2 ± 1.0 [a]
F1	29.4 ± 0.3 [a]	0.68 ± 0.02 [b]	45.4 ± 1.3 [b]
F2	28.7 ± 0.3 [a]	0.54 ± 0.01 [a]	53.8 ± 1.8 [c]
2014			
OF	22.4 ± 0.8 [a]	0.72 ± 0.02 [c]	34.9 ± 1.3 [a]
F1	36.2 ± 0.9 [c]	0.50 ± 0.01 [b]	74.8 ± 2.8 [b]
F2	32.0 ± 0.7 [b]	0.41 ± 0.01 [a]	75.0 ± 2.9 [b]
2015			
OF	31.1 ± 1.1 [b]	0.87 ± 0.05 [c]	40.8 ± 2.9 [a]
F1	24.1 ± 0.5 [a]	0.53 ± 0.03 [b]	49.5 ± 2.7 [a]
F2	23.6 ± 0.8 [a]	0.26 ± 0.01 [a]	95.9 ± 6.1 [b]

Values represent mean ± SE (n = 21–30); different letters denote statistically significant differences between different cropping systems for each year ($P < 0.05$).

In all 3 years, the highest accumulation of EpFlav was in the leaves of OF grown plants, compared to polytunnels with reduced PAR and UV radiation (Table 2). Moreover, the content of EpFlav was higher in the leaves of plants from F1 compared to those grown in F2, which received four times lower UV-A and almost no UV-B radiation. This is in line with numerous reports on induction of phenylpropanoids and flavonoids in the leaf epidermis by UV radiation (Schreiner et al., 2014; Vidović et al., 2017). Stimulated accumulation of flavonoids in the leaves can contribute to increased tolerance to strong sunlight and resistance to pathogens (Lattanzio et al., 2006; Agati et al., 2013). Moreover, lower NBI in the leaves of plants grown in the OF, compared with F1 and F2, corresponded to intraleaf allocation of resources towards flavonoid metabolism (Meyer et al., 2006).

3.2. Carotenoid accumulation in tomato fruits

Distribution of Ly in the tomato fruit was not uniform: its content was several times higher in the dried peel compared with the dried flesh, irrespective of radiation regimes (Figure 1). This is in line with results reported by Toor and Savage (2005) for three tomato cultivars. Lycopene was the major carotenoid in tomato and contributed about 80%–95% to total carotenoids in the peel and 70%–85% in the flesh, similarly to previously reported results (Dorais et al., 2008; Kotíková et al., 2011). Only in 2014 was β-Car lower in the peel compared to the flesh, and the portion of Ly in the flesh was about 62%, which can be attributed to extremely high precipitation and lower insolation in this year.

The content of Ly in the peel of tomato fruits showed more variation between the years than among the CSs (for significant effects see Table B2, Appendix B). On the other hand, higher accumulation of β-Car in the peel was observed in the fruits grown under polytunnels compared to the OF. In 2013 and 2015 (sunnier years than 2014) Ly and β-Car contents in the flesh were higher in fruits from the F1 polytunnel than F2, which transmitted 4 times lower UV-A and almost completely excluded UV-B radiation. It was shown that the effects of UV radiation on Ly and β-Car accumulation (stimulative or inhibiting) depend on intensity, duration, and quality of light (Giuntini et al., 2005; Dorais et al., 2008; Bian et al., 2015; Ilić et al., 2015). For example, Guintini et al. (2005) reported a positive effect of UV-B radiation on total Ly content in one tomato hybrid, while there was no effect in the other. Furthermore, Kläring and Krumbein (2013) reported a positive correlation of β-Car content and PAR in the whole tomato fruit, without affecting Ly content. On the contrary, in cherry tomato cultivar Alina, Ly content in the fruit was higher in the screenhouse, which had 30%–55% reduced PAR compared to OF, while β-Car content was unaffected (Leyva et al., 2014).

3.3. Phenolic compounds in tomato fruits

The main HBAs in tomato fruits were protocatechuic acid (PA), syringic acid (SA), and an unidentified HBA

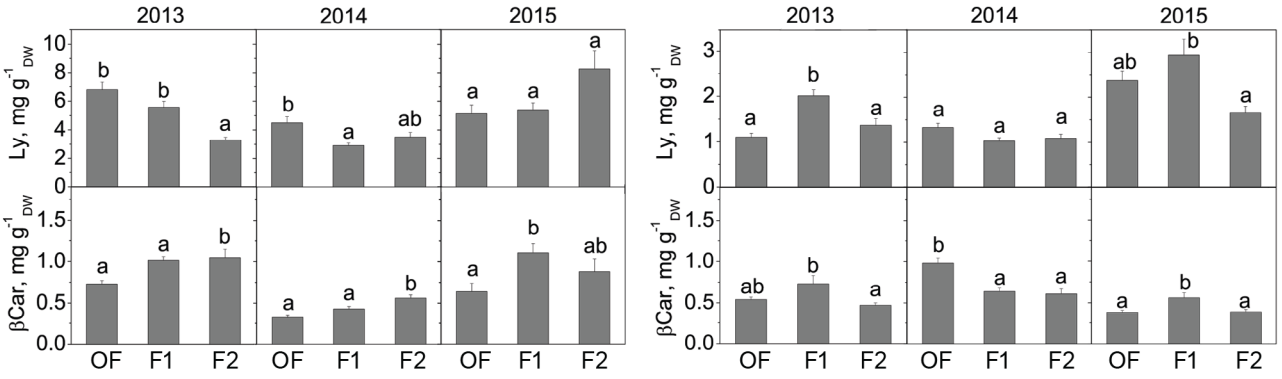

Figure 1. Content of lycopene (Ly) and β-carotene (β-Car) in the peel (left) and flesh (right) of tomato fruits grown in the OF and under two polytunnels (F1 and F2) during 2013, 2014, and 2015. Values represent mean ± SE (n = 4–5); different letters denote statistically significant differences between different CSs for each year for peel and flesh ($P < 0.05$).

derivative (spectral characteristics: peak at 264 nm, shoulder at 290 nm). The contents of the three HBAs in tomato peel and flesh were not affected by different CSs in 2013 and 2014 (Figure 2; significant Y and CS effects are given in Table B2 in Appendix B). However, in 2015 the peel content of PA and the unknown HBA derivative was higher in the OF than in the fruits grown in F1 and F2.

Derivatives of caffeic acid (CA), *p*-coumaric acid (*p*-CA), and ferulic acid (FA) were the most abundant HCAs in the fruits. Similar HCA composition was reported for other tomato cultivars (Luthria et al., 2006; Anton et al., 2014). In tomato peel the decrease in CA and FA content was in correlation with PAR reduction for all 3 years, since no significant changes were observed between F1 and F2 (Figure 3; for significant CS effects see Table B2 in Appendix B). No consistent trend in CA, *p*-CA, and FA content in the flesh of tomatoes regarding light conditions was found between the years. These results may suggest that preferential accumulation of CA and FA in the peel is a part of acclimation response to direct exposure to solar radiation, while in the flesh other factors may influence the composition of HCAs. Similarly to our results for 2013 and 2014, Calvenzani et al. (2015) reported that total concentration of HCAs in fully ripe tomato fruits was higher under ambient UV-B than in UV-B shielded fruits.

The main flavonoid aglycones in the Big Beef F1 cultivar were quercetin (Q), kaempferol (K), and chalconaringenin (ChN), which was consistent with the flavonoid composition of other tomato cultivars (Slimestad et al., 2008; Anton et al., 2014). Independently of the year and

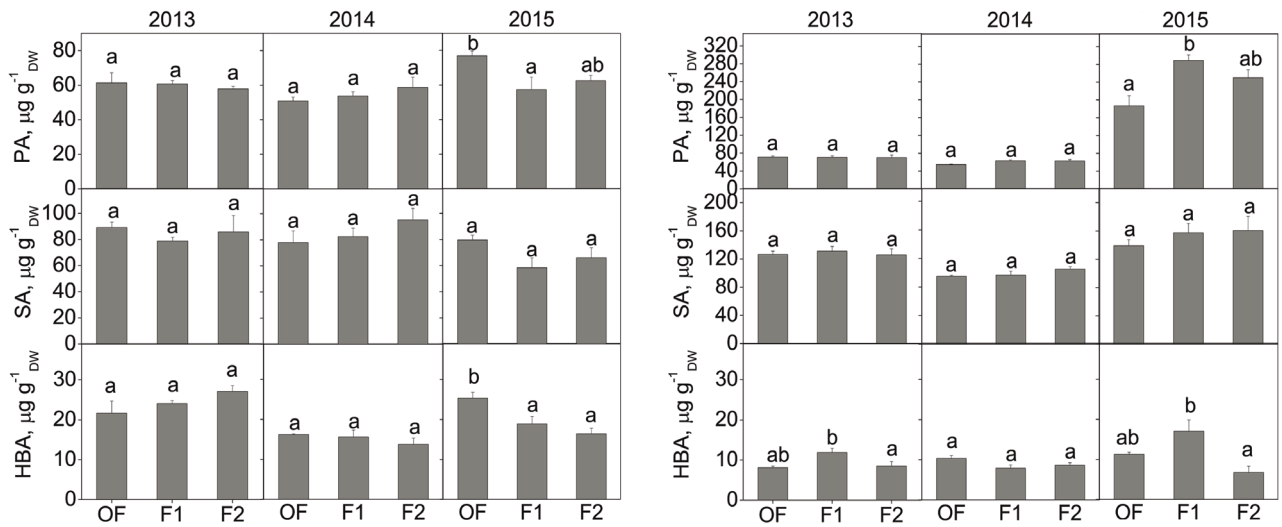

Figure 2. Contents of protocatechuic acid, syringic acid, and unidentified hydroxybenzoic acid derivative (PA, SA, and HBA) in the peel (left) and flesh (right) of tomato fruits grown in the OF and under two polytunnels (F1 and F2) during 2013, 2014, and 2015. Values represent mean ± SE (n = 4–5); different letters denote statistically significant differences between different CSs for each year for peel and flesh ($P < 0.05$).

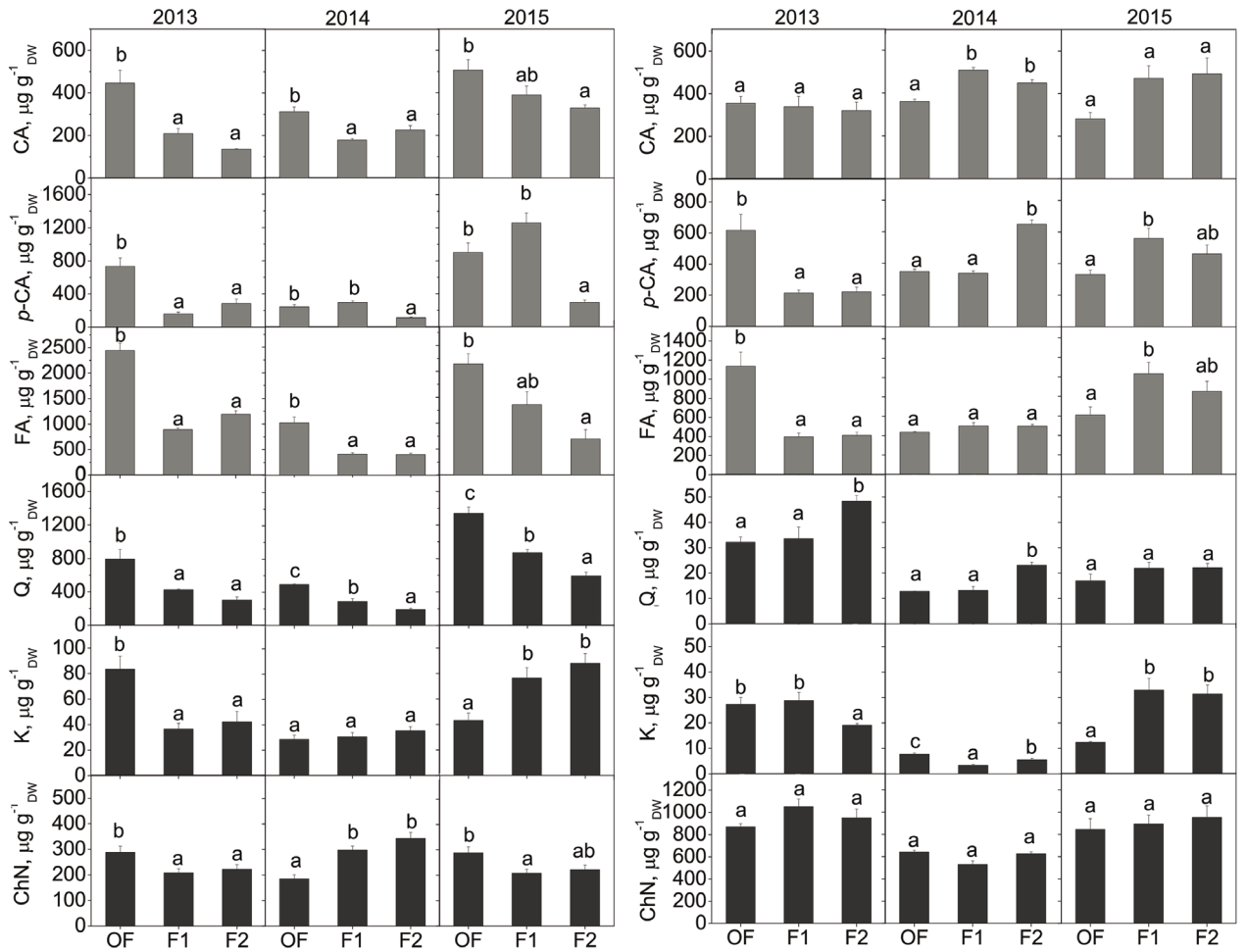

Figure 3. Contents of caffeic, *p*-coumaric, and ferulic acids (CA, *p*-CA, and FA) and quercetin, kaempferol, and chalconaringenin (Q, K, and ChN) in the peel (left) and flesh (right) of tomato fruits grown in the OF and under two polytunnels (F1 and F2) during 2013, 2014, and 2015. Values represent mean ± SE (n = 4–5); different letters denote statistically significant differences between different CSs for each year for peel and flesh ($P < 0.05$).

CS, both Q and K contents were higher in the peel than in the flesh, while the content of their biosynthetic precursor, ChN, was almost two times higher in the flesh in all 3 years (Figure 3). Moreover, in all 3 years, the content of Q was the highest in the peel of fruits grown in the OF and lowest in F2, implicating the role of PAR and UV-B radiation in stimulation of Q biosynthesis. This is in line with higher content of Q and its glycosides in the peel compared to the flesh of tomato grown under full solar radiation (Giuntini et al., 2008).

The HCAs and flavonoids with *ortho*-dihydroxyl substitution on the B-ring (e.g., Q, CA) are more efficient antioxidants than those with one hydroxyl group (K, *p*-CA) (Rice-Evans et al., 1997). During all 3 years, peel content of Q and CA was the highest in the fruits from the OF, while K and *p*-CA differentially varied with changes in PAR and UV radiation (significant CS effects are given in Table B2 in Appendix B). These results indicate enhancement of antioxidative capacity of the fruits. Increased HCA and flavonol accumulation (mostly Q glycosides) by full sun exposure was observed not only in tomato (Giuntini et al., 2008; Leyva et al., 2014), but also in the skins of other fruits, such as apples (Merzlyak et al., 2002) and grape berries (Martinez-Lüscher et al., 2014).

Cultivar-specific and annual variability in polyphenolic content (HCAs, flavonoids) in tomato fruits grown both in open fields and under polytunnels and greenhouses was reported (Chassy et al., 2006; Anton et al., 2014). The interaction of temperature and light quality, quantity, and duration is the most important factor affecting the nutritional value (phenolic and carotenoid content) of tomato (Dumas et al. 2003; Dorais et al., 2008).

3.4. Conclusions

According to our results, stimulation of beneficial phytonutrients in tomato fruits is determined not only by the intensity of solar radiation components, but by other variable weather conditions as well. In all 3 years of the study, PAR and UV radiation (both UV-A and UV-B) had a synergistic effect on the accumulation of dihydroxylated polyphenols such as CA and Q in the tomato peel. Furthermore, significant accumulation of EpFlav in the leaves of plants from the OF and in F1 (compared to F2) enhanced the overall plant resilience to environmental conditions during the ripening period. Finally, when comparing the two polytunnels, we showed that tomato fruits grown under the foil with higher UV transmittance (F1) had higher contents of *p*-CA and Q in the peel and Ly and β-Car in the flesh. Therefore, by choosing covering materials with higher UV-transmittance in tomato production, the antioxidative capacity of fruits can be improved without influencing fruit weight.

Acknowledgments

This work was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Project No. III 43010). The authors would like to thank Zorica Milošević for growing the tomatoes (Organic Food Produces, household Milošević Svilajnac).

References

Agati G, Brunetti C, Di Ferdinando M, Ferrini F, Pollastri S, Tattini M (2013). Functional roles of flavonoids in photoprotection: new evidence, lessons from the past. Plant Physiol Biochem 72: 35-45.

Anton D, Matt D, Pedastsaar P, Bender I, Kazimierczak R, Roasto M, Kaart T, Luik A, Püssa T (2014). Three-year comparative study of polyphenol contents and antioxidant capacities in fruits of tomato (*Lycopersicon esculentum* Mill.) cultivars grown under organic and conventional conditions. J Agric Food Chem 62: 5173-5180.

Avena-Bustillos RJ, Du WX, Woods R, Olson D, Breksa AP, McHugh TH (2012). Ultraviolet-B light treatment increases antioxidant capacity of carrot products. J Sci Food Agr 92: 2341-2348.

Becatti E, Petroni K, Giuntini D, Castagna A, Calvenzani V, Serra G, Mensuali-Sodi A, Tonneli C, Ranieri A (2009). Solar UV-B radiation influences carotenoid accumulation of tomato fruit through both ethylene-dependent and -independent mechanisms. J Agric Food Chem 57: 10979-10989.

Bian ZH, Yang QC, Liu WK (2015). Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. J Sci Food Agric 95: 869-877.

Boulard T, Raeppel C, Brun R, Lecompte F, Hayer F, Carmassi G, Gailard G (2011). Environmental impact of greenhouse tomato production in France. Agron Sustain Dev 31: 757-777.

Calvenzani V, Castagna A, Ranieri A, Tonelli C, Petroni K (2015). Hydroxycinnamic acids and UV-B depletion: profiling and biosynthetic gene expression in flesh and peel of wild-type and hp-1. J Plant Physiol 181: 75-82.

Cerović ZG, Masdoumier G, Ghozlen NB, Latouche G (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol Plant 146: 251-260.

Chassy AW, Bui L, Renaud EN, Van Horn M, Mitchell AE (2006). Three-year comparison of the content of antioxidant microconstituents and several quality characteristics in organic and conventionally managed tomatoes and bell peppers. J Agric Food Chem 54: 8244-8252.

Davuluri GR, Van Tuinen A, Eraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR., Palys J, Uhlig J et al. (2005). Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nat Biotechnol 23: 890-895.

Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A (2013). Dietary (poly)phenolics in human health: structures bioavailability and evidence of protective effects against chronic diseases. Antioxid Redox Signal 18: 1818-1892.

Dorais M, Ehret DL, Papadopoulos AP (2008). Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem Rev 7: 231-250.

Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci Food Agric 83: 369-382.

Eurostat (2016). Europe in Figures: EUROSTAT Yearbook 2016. Luxembourg City, Luxembourg: European Commission.

Giuntini D, Graziani G, Lercari B, Fogliano V, Soldatini GF, Ranieri A (2005). Changes in carotenoid and ascorbic acid contents in fruits of different tomato genotypes related to the depletion of UV-B radiation. J Agric Food Chem 53: 3174-3181.

Giuntini D, Lazzeri V, Calvenzani V, Dall'Asta C, Galaverna G, Tonelli C, Petroni K, Ranieri A (2008). Flavonoid profiling and biosynthetic gene expression in flesh and peel of two tomato genotypes grown under UV-B-depleted conditions during ripening. J Agric Food Chem 56: 5905-5915.

Gould KS, Lister C (2005). Flavonoid functions in plants. In: Andersen ØM, Markham KR, editors. Flavonoids: Chemistry Biochemistry and Applications. 1st ed. Boca Raton, FL, USA: CRC Press, pp. 397-441.

Grierson D, Kader A (1986). Fruit ripening and quality. In: Atherton J, Rudich G, editors. The Tomato Crop. 1st ed. London, UK: Chapman and Hall, pp. 241-280.

Ilić ZS, Milenković L, Šunić Lj, Fallik E (2015). Effect of coloured shade-nets on plant leaf parameters and tomato fruit quality. J Sci Food Agric 95: 2660-2667.

Jansen MAK, Hectors K, O'Brien NM, Guisez Y, Potters G (2008). Plant stress and human health: do human consumers benefit from UV-B acclimated crops? Plant Sci 175: 449-458.

Kläring HP, Krumbein A (2013). The effect of constraining the intensity of solar radiation on the photosynthesis, growth, yield and product quality of tomato. J Agron Crop Sci 199: 351-359.

Kotíková Z, Lachman J, Hejtmánková A, Hejtmánková K (2011). Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. Lebenson Wiss Technol 44: 1703-1710.

Lamnatou C, Chemisana D (2013). Solar radiation manipulations and their role in greenhouse claddings: Fresnel lenses, NIR-and UV-blocking materials. Renew Sust Energ Rev 18: 271-287.

Lattanzio V, Lattanzio VM, Cardinali A (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F, editors. Phytochemistry: Advances in Research. 1st ed. Trivandrum, India: Research Signpost, pp. 23-67.

Leyva R, Constán-Aguilar C, Blasco B, Sánchez-Rodríguez E, Romero L, Soriano T, Ruíz JM (2014) Effects of climatic control on tomato yield and nutritional quality in Mediterranean screenhouse. J Sci Food Agric 94: 63-70.

Luthria DL, Mukhopadhyay S, Krizek DT (2006). Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. J Food Compost Anal 19: 771-777.

Martínez-Lüscher J, Torres N, Hilbert G, Richard T, Sánchez-Díaz M, Delrot S, Aguirreolea J, Pascual I, Gomès E (2014). Ultraviolet-B radiation modifies the quantitative and qualitative profile of flavonoids and amino acids in grape berries. Phytochemistry 102: 106-114.

Meyer S, Cerović ZG, Goulas Y, Montpied P, Demotes-Mainard S, Bidel LPR, Moya I, Dreyer E (2006). Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbon-nitrogen balance within leaves? Plant Cell Environ 29: 1338-1348.

Merzlyak MN, Solovchenko AE, Chivkunova OB (2002). Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol Biochem 40: 679-684.

Neugart S, Zietz M, Schreiner M, Rohn S, Kroh LW, Krumbein A (2012). Structurally different flavonol glycosides and hydroxycinnamic acid derivatives respond differently to moderate UV-B radiation exposure. Physiol Plant 145: 582-593.

Olives Barba AI, Cámara Hurtado M, Sánchez Mata MC, Férnandez Ruiz V, López Sáenz de Tejada M (2006). Application of a UV-Vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. Food Chem 95: 328-336.

Olle M, Viršilé A (2013). The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agric Food Sci 22: 223-234.

Peet MM, Welles GWH (2005). Greenhouse tomato production. In: Heuvelink E, editor. Tomatoes - Crop Production Science in Horticulture, Vol. 13. 1st ed. Wallingford, UK: CABI Publishing, pp. 257-304.

Rao AV, Rao LG (2007). Carotenoids and human health. Pharmacol Res 55: 207-216.

Rice-Evans CA, Miller NJ, Papanga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci 2: 152-159.

Sabir N, Singh B (2013). Protected cultivation of vegetables in global arena: a review. Indian J Agr Sci 83: 123-135.

Schreiner M, Martínez-Abaigar J, Glaab J, Jansen MAK (2014). UV-B induced secondary plant metabolites. Optik Photonik 9: 34-37.

Slimestad R, Fossen T, Verheul MJ (2008). The flavonoids of tomatoes. J Agric Food Chem 56: 2436-2441.

Toor RK, Savage GP (2005). Antioxidant activity in different fractions of tomatoes. Food Res Int 38: 487-494.

Tremblay N, Wang Z, Cerović ZG (2012). Sensing crop nitrogen status with fluorescence indicators. A review. Agron Sustain Dev 32: 451-464.

Vidović M, Morina F, Milić S, Zechmann B, Albert A, Winkler JB, Veljović Jovanović S (2015). UV-B component of sunlight stimulates photosynthesis and flavonoid accumulation in variegated *Plectranthus coleoides* leaves depending on background light. Plant Cell Environ 38: 968-979.

Vidović M, Morina F, Veljović Jovanović S (2017). Stimulation of various phenolics in plants under ambient UV-B radiation. In: Singh VP, Singh S, Prasad SM, Parihar P, editors. UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth. 1st ed. Chichester, UK: Wiley-Blackwell, pp. 9-56.

Zhang H, Tsao R (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opi Food Sci 8: 33-42.

Appendix A.
Average monthly weather conditions during the experiments in 2013, 2014, and 2015.

Table A1. Average monthly weather conditions during the experiments in 2013, 2014 and 2015.

	Insolation, h	Precipitation, mm	Minimal temperature, °C	Maximal temperature, °C	Average daily temperature, °C	Relative humidity, %	Cloudiness
2013							
Feb	38.5	87.4	1.4	7.4	3.8	82	8.1
Mar	117.3	38.1	2.7	11.3	5.7	73	6.6
Apr	206.9	64.3	7.2	20.8	12.7	69	4.5
May	226.5	78.0	10.0	25.1	18.1	68	5.6
Jun	227.0	20.8	13.4	26.2	19.8	75	5.0
Jul	311.0	25.3	15.9	30.3	21.7	66	3.1
2014							
Feb	111.9	15.9	0.6	8.1	6.1	73	5.4
Mar	181.9	111.3	1.0	12.0	8.8	75	5.2
Apr	141.5	185.2	5.2	18.7	12.1	78	6.7
May	212.1	85.4	11.3	24.4	15.6	78	6.2
Jun	240.5	124.6	13.8	26.7	19.4	76	5.0
Jul	243.6	56.0	13.2	32.7	21.6	76	5.0
2015							
Feb	110.1	41.1	-1.3	12.6	2.6	78	5.4
Mar	119.9	46.3	2.0	16.0	6.5	76	6.6
Apr	224.1	115.1	4.0	18.2	11.2	65	5.1
May	213.3	80.3	11.4	22.2	17.6	73	5.1
Jun	244.0	21.5	13.2	26.4	19.7	73	4.7
Jul	333.1	26.7	15.7	28.8	24.4	60	2.3

Appendix B

Statistical analysis (two-way ANOVA) for the effects of cropping system (CS) and year (Y), and their interactions on NBI, and on the contents of Chl, EpFlav, carotenoids, and phenolics in the leaves of tomato grown in the open field (OF) and under two polytunnels (F1 and F2) during 2013, 2014, and 2015 are shown in Tables B1 and B2.

Table B1. Two-way ANOVA results for the effects of CS and Y and their interactions on the contents of Chl and EpFlav, and NBI, in the leaves of tomato plants.

Trait	CS	Y	CS × Y
Chl	<0.001	<0.001	<0.001
EpFlav	<0.001	<0.001	<0.001
NBI	<0.001	<0.001	<0.001

Table B2. Two-way ANOVA results for the effects of CS and Y and their interactions on the contents of phenolics compounds in the peel and flesh of tomato fruits.

Trait	Peel			Flesh		
	CS	Y	CS × Y	CS	Y	CS × Y
Ly	0.3085	<0.0010	<0.001	<0.0010	<0.0010	<0.0010
β-Car	<0.0010	<0.0010	0.0928	<0.0010	<0.0010	<0.0010
PA	0.2295	0.0033	0.0265	0.0015	<0.0010	<0.0010
SA	0.6383	0.0063	0.1131	0.3470	<0.0010	0.7814
HBA	0.1414	<0.0010	0.0031	<0.0010	0.0311	<0.0010
CA	<0.0010	<0.0010	0.0167	0.0046	0.0106	0.0303
p-CA	<0.0010	<0.0010	<0.001	0.0051	0.0242	<0.0010
FA	<0.0010	<0.0010	0.0033	0.1045	<0.0010	<0.0010
Q	<0.0010	<0.0010	<0.001	<0.0010	<0.0010	<0.0010
K	0.7946	<0.0010	<0.001	0.0259	<0.0010	<0.0010
ChN	0.0013	0.1069	<0.001	0.4057	<0.0010	0.2835

Ly, Lycopene; β-Car, β-carotene; PA, protocatechuic acid; SA, syringic acid; HBA, hydroxybenzoic acid; CA, caffeic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; Q, quercetin; K, kaempferol; ChN, chalconaringenin.